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RESEARCH PAPER

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Relation between Maternal Haematocrit and Serum Iron Concentration in Different Stages of Pregnancy

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ABSTRACT

Iron deficiency anaemia accounts for 60% of nutritional anaemia in pregnancy. The prevalence of iron deficiency is far greater than the prevalence of anaemia in general and iron deficiency often develops during the later stages of pregnancy even in women who enter pregnancy with relatively adequate iron stores and this may not reflect on the haematocrit immediately. In this study, 80 pregnant women were recruited for assessment; 20 pregnant women in the first trimester, 30 in the second trimester and 30 in the third trimester. Dual blood samples (EDTA anticoagulated and coagulated blood samples) were collected, Haematocrit / packed cell volume, serum iron and blood film

examination was conducted by standard methods. The results showed mean haematocrit of 0.34 ± 0.038 in the first trimester, 0.34 - 0.032 in the second trimester and 0.32 ± 0.035 in the third trimester. The mean serum iron level in the first trimester was 109.3 ± 30.4, 104.3 ± 28.2 in the second trimester and 86.8 ± 25.5 in the third trimester. There were no significant differences p>0.05 in the results except decreased serum iron of 86.8 ± 25.5 in the third trimester (p<0.05 l and 2 vs 3). This study showed that haematocrit/ Packed Cell Volume was normal in all the trimesters but serum iron showed significant decrease as pregnancy stages progressed.

Keywords: Maternal Haematocrit, Serum Iron Concentration and Pregnancy.

INTRODUCTION

In recent times, it has been observed that the rate of maternal mortality is on the increase. In sub-Saharan Africa particularly Maiduguri metropolis, complication of pregnancy is a common feature in hospitals (Iloabachie and Meniru, 1990). This problem has warranted a special interest of the Federal Ministry of Health (FMOH). One of the problems that can lead to morbidity and mortality in pregnant women is anaemia of pregnancy. An association of anaemia with maternal and perinatal mortality is well established and in developing countries severe anaemia contributes directly or indirectly to the high incidence pf maternal deaths (Mitchell, 2002). Maternal risk factors such as anaemia and pregnancy induced hypertension commonly predispose to preterm delivery and low birth weight; There is an alarmingly high mortality in this population (Arvind, 2004).

Anaemia in pregnancy is defined as the significant reduction in circulating haemoglobin or red cell mass below the critical level in pregnancy as compared with pregnant women of the same age and geographical location. A high proportion of women in both industrialized and developing countries become anaemic during pregnancy. Estimates from the World Health Organization report that from 35% to 75% (56% on average) of pregnant women in developing countries, and 18% of women from industrialized countries are anemic. A prevalence study carried out in South Eastern Nigeria (Adinma, 2002), showed the prevalence of anaemia in pregnancy to be 40.4%. About 4-16% of maternal death is due to anaemia. In Nigeria, it is responsible for 11.0% of maternal deaths (Anorlu *et al*., 2006). It also increases the maternal morbidity, foetal and neonatal mortality and morbidity significant.

In West Africa, anaemia in pregnancy results from multiple Causes, including iron and folate deficiency (nutritional deficiencies); malaria and hookworm infestation and infection; other infections, such as HIV; and haemoglobinopathies. It is well known that the maternal nutritional status during pregnancy affects neonate birth weight and growth especially in the early years of life (Hardy et al., 2004). Iron and folate deficiency is by far the most important aetiologic factor; iron deficiency anaemia accounts for 60% of nutritional anaemia in pregnancy. It is therefore believed to be the most common cause of anaemia in pregnancy (Nyuke, 2000). About one in five women develop iron deficiency anaemia when they are pregnant. Iron deficiency anaemia can have negative consequences for pregnant women (Vazirinejad *et al.*, 2007). Iron deficiency among pregnant women can result in mortality and also in premature birth and low birth weight. A 2003 nutritional survey (Maziya-Doxion *et al.*, 2004) found that about 20% of pregnant women suffer from iron deficiency (WHO, 2005).

In pregnancy, the gestational period is divided into the first, second and third trimesters. To obtain iron balance in early pregnancy, only the basal iron requirement (0.8mg/day or

6% of the daily dietary iron intake) is needed (Barrett et al., 1994). Extra iron is required for expansion of red cell mass in the second trimester while more iron is needed for the developing foetus and placenta in the third trimester. Hence, if no changes occur in the iron store, then the daily absorption of the dietary iron would need to increase from 6% to 32% (3.Sing/clay) over the last 28 weeks of pregnancy (Barrett et al., 1994). Iron requirements are highest in the second and third trimesters of pregnancy (Lynch, 2000). In this part of the countrv however, most women enter pregnnacy with depleted iron stores, some pregnant women do not obtain the basal iron requirements from their diets and many have depleted iron stores irrespective of the trimester (probably because they do not space children adequately). Such a study that will present data on serum iron and haematocrit relation in the different trimesters is yet to be conducted in this part of the country. Therefore, this study will give basal data for future analysis and related researches.

OBJECTIVES OF THE STUDY

1. To assess or screen haematocrit and serum iron in different stages (trimesters) of pregnancy.
2. To relate serum iron to the haematocrit of the patients.
3. To deduct any other information that may be adduced from the study.
4. To relate the haematological findings to their age and weight.

SUBJECTS AND MATERIALS

SUBJECTS

Pregnant women diagnosed biochemically and clinically, and seen at the antenatal clinics of the University of Maiduguri Teaching Hospital (U.M.T.H.) were recruited into the study. The women not diagnosed by these criteria were excluded. (The biomedical diagnosis is by testing for the presence of Human Chorionic Gonadotrophic hormone in urine, carried out in the Chemical Pathology department of the hospital; and the clinical diagnosis by scanning carried out in the Radiology department of the hospital ĸ

STUDY AREA

The study was conducted at the University of Maiduguri Teaching Hospital, Maiduguri. Maiduguri is the capital of Borno state and is situated at the North Eastern part of Nigeria. The city lies within sheet 90NE of the federal survey of Nigeria. The city lies within the latitude 11°48N to 1T55N and 130°4'E to 13°1'E. Borno State has an area of 61,435 square kilometers aiid is the largest state in Nigeria in terms of land mass. Within the country,its neighbours are Adamawa State to the South, Yobe State to the west and Gombe State to the southwest. The state equally shares borders with countries such as the Republics of Niger to the north, Chad to the northeast and Cameroon to the east. Based on the 2006 provisional census figures, Borno State has a population of 4,151,193 and population density of approximately 60 inhabitants per square kilometers. The area is generally accessible by road. Most of the roads are tarred while few are foot paths. Maiduguri is quite heterogeneous in ethnic groups. The Kanuri is the dominant ethnic group and accounts for about three quarters of the the Maiduguri populace. Other ethnic groups are bura, Babur, Marghi, Fulani, Hausa, Kanukuru, Gamergu, Chibok, Ngoshe, Guduf, Mandara. Tera and several other smaller groups (Wilhelm *et al.*, 1983). The type of food they eat include maize, millet, guinea corn, rice, beans, fish, beef meat, camel meat, yam, cassava, orange guava, mango, etc. Borno state has a climate which is hot and dry for a greater part of the year

although the southern part is slightly hotter. University of Maiduguri Teaching Hospital was established in (1975) in fulfilment of the vision to improved Health care de liven- system in North Eastern part of the country.

MATERIALS AND REAGENTS

MATERIALS FOR SAMPLE COLLECTION

The materials used for haematocrit measurement included the following:

- Syringe and needle
- Cotton wool
- 75% alcohol
- Tourniquet
- Ethylenediaminetetracetic acid (EDTA) anticoagulant bottles
- Plain blood bottles

MATERIALS FOR HAEMATQCRIT MEASUREMENT

The materials used for haematocrit measurement included the following:

- Heparinized capillary tube
- Sealant- plasticine, Bunsen flame
- Microhaematocrit centrifuge
- Microhaematocrit reader

MATERIALS FOR SERUM IRON ESTIMATION

The materials used for serum iron estimation included the following

- Glass test tubes
- Microtitre pipette and pipette tips
- Distilled water
- Serum iron reagent
- Cuvette

MATERIALS FOR FILM PREPARATION. STAINING AND EXAMINATION

The materials used for serum iron estimation included the following

- clean grease free slide
- Spreader
- Leishman stain
- Light microscope
- Glass cover
- Cotton wool
- Cedar wood oil immersion
- Buffered water pH 8.6
- Staining rack

METHODOLOGY

SAMPLE COLLECTION

After filling the questionnaire completely, the blood sample was collected from the patients. The most commonly used site for collection of the sample was vene puncture at the (antecubital fossa) i.e. the vein inside the bend of the elbow. A tourniquet was applied to the upper arm, sufficiently tight to restrict the venous flow and make the veins stand out.

The patient was ask to keep the arm straight and clench the fist, the suitable vein was selected and the site swabbed with 75% alcohol, then allow to dry for some seconds while the syringe and containers (EDTA and Plain blood bottles) were prepared appropriately for use. The needle was inserted smoothly with the bevel facing upward at an angle of 20°-30° to surface of the arm and in a direct line with the vein. After the needle entered the vein, blood was withdrawn into the syringe and tourniquet was released after sufficient quantity of blood was collected. A piece of dry cotton wool was place at the puncture sight as the needle was gently removed and pressed to stop the flow of blood. The patient was asked to release the clenched fist and the blood was dispensed in the plain blood bottle and EDTA bottle (mixed) evenly (Ochei and Kolhatkar, 1998).

The EDTA blood sample was used to run Packed Cell Volume and blood film preparation for blood picture examination, while the plain (coagulated) blood sample was centrifuged and the serum used to investigate serum iron levels.

Precautions: Sterile EDTA containers were used to collect the sample to avoid introduction of contaminants. Each patient was bled carefully by vene puncture to avoid accidental needle prick. Hand gloves and laboratory coat were used for protection. Ail EDTA samples were analysed immediately after collection without delay to avoid remission errors.

DETERMINATION OF PACKED CELL VOLUME

PRINCIPLE

When anticoagulated whole blood in a glass capillary tube of specific diameter and length, is centrifuged in a microhaematocrit centrifuge at a known speed (12000G) and known time (5minutes) to obtain constant packing of the red cells; the volume of the packed red cells and percentage of the whole blood are determined by a special haematocrit reader.

PROCEDURE

The heparinized capillary tube was filled up to two thirds of its length with well mixed whole blood and the unfilled end sealed using a Bunsen flame rotating the end of the capillary tube in the flame. The fiiied capillary tube was placed in the microhaematocrit centrifuge and spun for 5minutes at 12,000g. The measurements were then read using the microhaematocrit reader.

Calculation PCV =

Packed RBC column height/ Total blood column height

Precautions: the EDTA blood sample was mixed properly before use to avoid erroneous results. The capillary tube was properly sealed to avoid spillage of blood during centrifugation.

SERUM IRON ESTIMATION

PRINCIPLE

Iron (III) reacts with chromazurol B (CAB) and Cetyltrimethyl amoniumbromide (CTMA) to form a coloured ternary complex with an absorbance maximum at 623nm. The intensity of the colour produced is directly proportional to the concentration of iron in the sample. A commercial kit (Human GmbH, Germany) based on this principle was used. The reagents were ready for use.

PROCEDURE

50ul of the sample (50ul of distilled water for the reagent blank) was added to 1ml of the reagent and mixed in a chemically clean test tube. It was allowed to incubate for 15minutes at 20-25°C and later the absorbance of the sample and the standard were measured against the reagent blank within 60minutes, at 623nm.

The concentration of iron in the sample was then calculated.

Precautions: To avoid contamination, the laboratory wares used were iron free (by using only disposable materials during the test). The distilled water was absolutely iron free. Turbid or haemolytic sera were not used.

FILM PREPARATION, STAINI AND EXAMINATION MAKING OF THIN BLOOD FILM

A thin blood film was made on each sample collected from each patient.

PRINCIPLE

Thin blood film is produced from an anticoagulated blood on a slide, smeared with a spreader to obtain less overlapping blood cells for microscopic examination.

PROCEDURE

A drop of anticoaguiated whole blood was placed at the edge of a clean grease-free glass slide. A spreader was positioned in front of the drop of blood, then pushed back to touch it. The blood was allowed to spread along the contact line of the spreader at an angle of 45 .The spreader was pushed forward smoothly and

Rapidly maintain the contract between the slide and the spreader

The smear made has head, body and tail. The thin film was allowed to air dry, labelled, then stained.

Precautions: the EDTA blood sample was mixed properly before use to ensure proper blood film representation of the samples. The blood films were not too thin or too thick to ensure reliable differential leucocyte counts and blood film examination.

STAINING OF THIN BLOOD FILM

Leishman stain is one of the Romanosky stains. It has remarkable properties and contains two dependent components i.e. Azure B (trimethyl thionin) and eosin Y (tetrabromoflourescein) dissolved in absolute methanol.

PRINCIPLE

The methanol is used fixed the film; eosin Y stains the basic component while Azure B stains the acidic component of the cells. The differential uptake of the stain by the cell is used to distinguish them.

PROCEDURE

The labelled thin blood film was arranged on the staining rack. The thin film was flooded with Leishman stain and allowed to fix for 2minutes. 8.6 pH buffered water was used to dilute and allowed to stain for Smmutes. The stained film was rinsed; back of the slide was wiped, air-dried, and then examined microscopically.

BLOOD FILM EXAMINATION AND LEUCOCYTES DIFFERENTIAL COUNT

The blood film made for differential leucocyte count was done according to the description of Dacie and Lewis (2008). An oil immersion was applied to the stained blood film and examined under the light microscope with XI00 objective lens. Longitudinal strip method was used to count each leucocyte cell against a 100 total count. Detection of abnormalities in leucocyte morphology was carried out carefully. The differential count were expressed as the

percentage of each type of cell and related to the total leucocyte counts. The general morphology of the efytlifocytes (red blood cells) was examined and abnormalities were duly reported.

RESULTS

Over the study period of 9 months, a total of 80 pregnant women were randomly recruited for the research. From each patient a sub structured questionnaire was filled and two blood samples collected (EDTA blood sample and plain blood sample). The samples collected were grouped according to trimester. 20(25%) pregnant women in their 1st trimester, 30(37.5%) in their 2nd trimester and 30(37.5%) in their 3rd trimester.

Table 1. Distribution of Patients by Age and Trimester.

AGE	TRIMESTER			TOTAL
RANGE	1 ST	I*11*	3**°	
(Years)				
17-19	2	1	1	4
20-29	15	22	2	58
30-39				16
40- 49	1	1		2
GRAND TOTAL	20	30	30*	80

Table 2. Haematological Results of the Pregnant Women Studied.

TRIMESTER	PCV	p Value	SERUM	p Value
	(L/L)	(PCV)	IRON	(IRON)
	Mean ± SD		(µg/dl)	
			Mean ± SD	
I3t(n = 20)	0.34 ± 0.038	p>0.05	109.3 ± 30.4	p>0.05
		(1 vs 2)		(1 vs 3)
2nd (n = 30)	0.34 ± 0.032	p>0.05	104.3 ± 28.2	
		(2 vs 3)		
3rd(n = 30)	0.32 ± 0.035	p>0.05	86.8 ±25.5	p<0.05
		(I vs 3)		(2vs3)

KEY

vs= versus.

Table 3. Haematological Results of all the pregnant women put together.

GROUP (n=80)	PCV(L/L) Mean ±SD	SERUM IRON (µg/dl) Mean ±SD
Patients	0.33 ±0.035	100.1±28.0

Table 4. Blood Picture Pattern of the Pregnant Women Studied.

Morphological Description	1ST (n=20)	2ND (n=30)	3RD (n=30
Normocytic	12(60%)	17(56.67%)	12(40%)
Normochromic	12(60%)	17(56.67%)	12(40%)
Microcytic	6(30%)	9(30%)	5(16.67%)
Hypochromic	4(20%)	8(26.67%)	6(20%)
Schistocytes	-	-	2(6.67%)
Acantocytes	-	1(3.33%)	1(3.33%)
Elliptocytes	3(15%)	-	4(13.33%)
Anicytoctyes	-	-	1(3.33%)
Poikilocytes	-	1(3.33%)	1(3.33%)

Table 5. Mean Differential Leucocyte Count of the Pregnant Women Studied.

WHITE CELL TYPE	PERCENTAGES		
	1ST Trimester	2ND Trimester	3RD Trimester
Neutrophils	67.8	68.3	64.1
Lymphocytes	31.2	29.5	34.3
Eosinophils	07	1.5	0.9
Monocytes	0.2	0.7	0.6
Basophils	0.1	-	-

Table 6. Relationship of Weights of Patient PCV and Serum Iron Values.

WEIGHT RANGES (Kg)	PCV L/L Mean±SD	SERUM IRON (µg/dl) Mean±SD
46-65(n=32)	0.34± 0.030	98.5±25.5
66-85(n=42)	0.32±0.040	99.8±30.7
86-105(n=6)	0.35±0.052	82.2±28.6

Table 7. Relationship of Heights of Patient PCV and Serum Iron Values

HEIGHT RANGES (Meters)	PCV L/L Mean±SD	SERUM IRON (µg/dl) Mean±SD
<1.50(n=1)	0.25	126.9
1.51-1.60(n=34)	0.34±0.031	98.0±27.8
1.61-1.70(n=35)	0.33±0.036	100.2±29.9
>1.70(n=10)	0.34±0.038	85.4±26.3

Table 8. Relation of Patient's Parity to PCV and Serum Iron Values.

PARITY	PCV L/L Mean±SD	SERUM IRON (µg/dl) Mean±SD
Primigravida(n=27)	0.34±0.032	93.6±23.9
1-2(n=30)	0.34±0.035	99.7±31.9
3-6(n=18)	0.32±0.039	99.5±26.8
7-11(n=5)	0.34±0.038	103.9±42.9

Table 9. Relation of Patient's Age Groups to PCV and Serum Iron Values.

AGE GROUPS (Years)	PCV L/L Mean±SD	SERUM IRON (µg/dl) Mean±SD
17-19(n=4)	0.35±0.017	86.8±35.2
20-29(n=58)	0.34±0.037	97.1±27.4
30-39(n=160)	0.33±0.037	97.7±27.7
40-49(n=2)	0.31±0.049	114.8±67.7

In this study,pregnant women in the first trimester (n=20) had mean PCV of 0.34 ±0.04 and serum iron level of 109.3±30.4 µg/dl.This serum iron level is with reference range of 37-145 µg/dl.This could be so because of the existing iron and haematinics supplementation they

receive. The finding of normal PCV and serum iron levels in the pregnant women in first trimester is in disagreement with findings elsewhere (Pathak et al., 2007). The appreciable serum iron level of the pregnant women reflected in normal Packed Cell Volume.

The women in the second trimester of pregnancy (n=30) had mean PCV of 0.34±0.03 and serum iron of 104.3± 28.2 µg/dl. The women in this group had slighlty lower iron value than those in the first trimester apparently due to higher physiologic demands by foetuss.It is known (Barret et al.,1994) that extra iron is required for red cell expansion in the second trimester. In these two cases PCV appeared unaffected because the development of signs and symptoms of iron deficiency is a sequential process taht does not show PCV change early. Pregnant women in the third trimester in this study had PCV of 0.32±0.04 and serum iron of 86.8± 25.5µg/dl. No significant differences in the results between the different (trimesters) groups were found for PCV but not for serum iron which discriminated against those in the third trimester.

It is well known that maternal nutritional status during pregnancy affects birthweight and growth,especially in the early years of life (Hardy et al.,2004). In Nigeria, data on status of iron,folate and vitamins B_{12} deificency are limited. This is also so in developing nations of the world.

When the haematological results of all the pregnant women are put together, the values were PCV 0.33±0.04 and serum iron 100±28 µg/dl. These prevailing values for the entire group are lower than the values recorded for the third trimester. Not much is known about the biological pathways by which matenal iron status may affect foetal metabolism and growth (Rasmussen and Stoltzfus, 2003). However, it has been established that as pregnancy progress, iron requirements for foetal growth rise steadily with growth of the foetus and iron requirement may reach as much as 10mg/day during the last 6-8 weeks orf pregancy. It is obvious that daily iron requirement cannot be met from dietary absorption alone in third trimester,even from an optimal diet (Kumar, 20080. Coupled with the known fact thatwomen enter preganncy with little or no iron stores (Dacie et al., 2008), this can account for the significant lower value of the third trimester serum iron levels, to that of the entire group.

When the red cell morphological blood picture results of the patients in each trimester were compared,the highest percentage of Normocytic Normochromic blood picture (60%) was recorded in the first trimester, 56% in the second trimester and 40% in the third trimester. This may probably be attributed to the low demand on the mother by the foetus 15% of the first trimester patients had elliptocytes. This can be as a result of dyserythropoietic activity due to effects of pregnancy (Kumar, 2008). Monocytic hypochromic blood picture occured more (30/26.67%) in the second trimester, than in the first and third trimesters.Though these findings do not reflect the iron status of the groups;this resultis due to wider outliers iron results in the second trimester. The iron-deplete status of the patients was aptly reflected in thier blood picture as the presence of schistocytes,Eliptocytes,anisocytosis and poikilocytosis were observed in the third trimester (even though they were minor occurences).

The comparism of the mean differential leucocyte count of the pregnant women studied in the trimesters showed no significant variations in their differential pattern.Neutrophils were the predominant cells, followed by lymphocytes, Eosinophils, Monocytes, and Basophils. There is limited data that relates iron status to differntial leucocyte count of pregnant women; therefore this cannot be further elaborated on.

When PCV and serum iron values were related to weight ranges, there were no significant

differences in PCV values across the weight groups.However,there was a significant decrease in the mean serum iron value (82.2± 28.6) of the 86-105Kg group as compared to the values of the 46-65 and the 66-85Kg group. In this study, it was not possible to measure the weight of the foetus;therefore the effect of weight on haematological parameters cannot be duly assessed. Neverthless, this weight group is the highest (86-105Kg) and individuals of this weight range are termed obese; the weight gain could increase the physiological demand for iron thereby resulting in the low serum iron values recorded.

The patern of results for PCV and serum iron in relation th the height of the pregnant women studied is dissimilar and generally contrasting in nature. The PCV and serum iron values appear to be independent of height as results did not show any consistent pattern in relation to height.

Relation of the haematological results to parity of the pregannt women studied revealed no significant differences in the haematocrit values among the groups. On the other hand,this was contrary for mean serum iron levels which branded the primigravidae group as having the lowest value (93.6 ±23.9) among the group. This could be a result of no previous iron supplementation, as is common with the multigravidae. The 7-11 births parity range possessed the highest serum iron level (103.9 ±42.9) among the groups. This information cannot be used objectively in conclusion due to the presence of outliers that escalate the mean values.

Apparently, age groups had little to no effect on the haematocrit or the serum iron results. This could be resultant of the few patients in the outer age cluster 17-19 (n=4) and 40-49 (n=2) which make comparism to the 20-29 (n=48) not objective. Age group 17-19 years has the lowest serum iron value (86.8 ± 35.2). This is in accordance with other studies conducted on adolescent girls (Toteja et al., 2006).

CONCLUSION

In this study,haematocrit was not affected in any stage of pregnancy but serum iron depleted as the pregnancy stages progressed. Regular iron supplementation will be beneficial to pregnant women.

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